cooling; chilling produces naturally the opposite effect (see Table 6). Other things being equal, the greater the degree of adsorption of calcium phosphate by the complex, the more rapidly will the system coagulate when acted upon by rennet. The enzymic attack proper is not, however, concerned in these changes, which appear instead to influence the renneting time mainly, if not exclusively, by their effect on the second or so-called calcium ion precipitation stage. Severe heat treatment, of course, can independently affect the sensitivity of the caseinate molecule to rennet enzyme attack and retard the coagulation as a whole. This, however, is apparently an additional effect of heating and does not in general mask the hysteresis phenomenon unless it is so severe as to inhibit coagulation altogether.

It is difficult to reconcile these results with the view that the enzymic part of rennet action consists of an alteration of a constituent of the original casein which acts, before this alteration, as a protective colloid for the main cation-sensitive group of constituents (Linderstrøm-Lang, 1928; Holter, 1932). Adsorption of calcium phosphate from solution by the caseinate complex as a result of heat treatment seems to affect the second stage (while leaving the first stage of the reaction unaffected) in much the same way as if the adsorption had occurred during this stage and subsequent to the enzyme reaction proper. This behaviour appears to conform with the older Hammarsten view of rennet action.

As to the differences observed in the type of hysteresis exhibited by caseinate-phosphate-gelatin systems according to whether caseinate or gelatin is the protecting colloid, the facts are best explained by assuming that the caseinate-phosphate complex is essentially a calcium caseinate-phosphate, i.e. a compound in which the union of phosphate and caseinate has taken place through calcium atoms common to both. The gelatin-protected phosphate would, on the other hand, appear to resemble a gold sol, and to consist presumably of a calcium phosphate nucleus coated with gelatin. Obviously the composition of the postulated caseinate-phosphate will not be fixed, but can vary according to the number of phosphate groups present. Moreover, the heterogeneity of casein is not relevant in either case; there is no suggestion that all the casein fractions are equally capable of forming these complexes.

SUMMARY

1. The rennet hysteresis of heated milk arises from the adsorption of calcium phosphate by the calcium caseinate-calcium phosphate complex during heating, followed by its gradual release again at lower temperatures.

2. Calcium phosphate thus adsorbed affects the renneting time primarily through its acceleration of the second or so-called calcium ion precipitation stage of the coagulation. This result seems to accord best with the Hammarsten theory of rennet action.

3. Rennet hysteresis of calcium caseinate-calcium phosphate-gelatin systems differs according to whether the phosphate is protected by caseinate or by gelatin. This behaviour is interpreted as favouring the existence of a chemical union between the two constituents in the calcium caseinate-calcium phosphate complex of milk.

I wish to thank Prof. J. J. McHenry for advice on the conductometric technique and Mr D. B. O'Loughlin for assistance in some of the experimental work.

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The Distribution of Myrobalanitannin

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(*Received* 15 *February* 1945)

Myrobalanitannin (I: luteoic acid 5-biglucoside, $R = C_{12}H_{21}O_{10}$) present in myrobalans, the fruit of *Terminalia chebula* Retz., was first obtained by Nierenstein (1910) as a well-crystallizing substance which gave, on hydrolysis either with dilute sulphuric acid or with emulsin, 1 mol. ellagic acid (II) and 2 mol. glucose. The hydrolysis with emulsin excludes the possibility that myrobalanitannin is

an acyl derivative (Fischer & Bergmann, 1918) but not the possibility that it is a diglucoside (e.g. III: luteoic acid 4:5-diglucoside, $R = R_1 = C_6H_{10}O_5$), and we have therefore methylated myrobalanitannin with diazomethane and hydrolyzed the product with dilute sulphuric acid. We obtained tetramethylellagic acid (IV), which thus confirms the original structure (I).

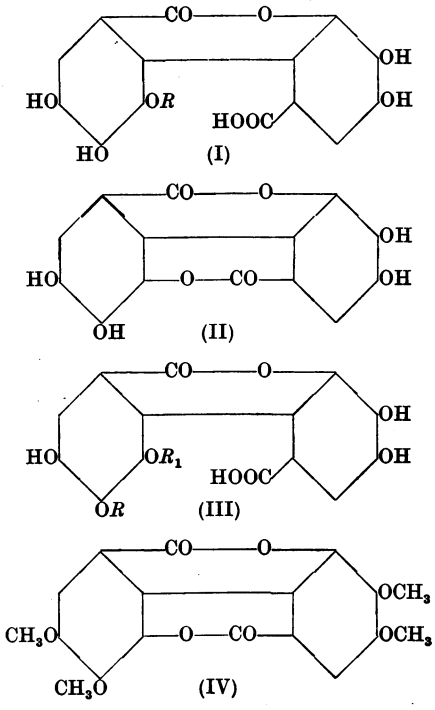
Table 1. *Isolation of myrobalanitannin from various sources*

	Yield (%)	Country of origin	Official name	Local name	Analysis* Glucose (%)	Analysis* Ellagic acid (%)
(i) Roots, rhizomes or underground stems:						
Ailanthus excelsa Rxb.	2·4	India	—	—	49·6	48·3
Donabanga mollucana Blr.	1·2	Java	—	—	49·5	48·4
Geranium maculatum L.	1·8	U.S.A.	—	—	49·7	48·6
G. wallachium Don.	2·0	France	—	—	49·3	48·7
Nupher luteum Sib. & Sm.	2·2	France	—	—	49·5	48·6
Nymphaea alba L.	0·5	Asia Minor	—	—	49·3	48·6
N. adorata Ait.	1·4	U.S.A.	—	—	49·4	48·6
Statica brasiliensis Bois	0·9	Chile	*Radix Bayeura*	—	49·2	48·3
St. caroliniana Willd.	1·1	Venezuela	—	—	49·3	48·2
St. Gmelinii Willd.	3·0	Caucasus	—	—	49·5	48·7
(ii) Barks:						
Ailanthus glandulosa Desf.	0·9	Japan	—	—	49·3	48·6
Aralia spinosa L.	1·81	U.S.A.	—	—	49·5	48·5
Aulomyrica ramulosa Bag.	1·1	Brazil	—	—	49·3	48·6
Companesia guaviroba† B. & H.	2·2	Paraguay	—	—	49·7	48·6
C. aphyla James & Nies.	1·71	U.S.A.	—	—	49·6	48·3
Daniella thurifera Beum.	1·98	Gold Coast	—	—	49·3	48·7
Eugenia opiculata DC.	3·2	Venezuela	—	—	49·5	48·2
E. jambolana Lam.	1·2	Mauritius	*Cortex Sygii Jambolanii*	—	49·2	48·5
Punica granatum L.	3·2	Egypt	*Cortex granatum*	—	49·4	48·7
Somodera indica Gaerth.	1·9	Ceylon	—	—	49·5	48·3
Sacrocephalus esculentus Afz.	5·4	Togoland	*Lignum Njimo*	Doundaká	49·3	48·5
Terminalia chebula Retz.	6·3	India	*Cortex Myrobalanii*	—	49·5	48·7
T. angustifolia Douglas & Nies.	2·5	Northern Rhodesia	—	—	49·4	48·4
Woodfordia floribunda Salisb.	11·8	India	—	—	49·5	48·3
W. siderafolia Nies.	1·5	Uganda	—	—	49·6	48·7
Rhizophera Ikotae Nies.	2·9	Northern Rhodesia	—	Majunji	49·3	48·5
(iii) Leaves:						
Acrostaphyllos uva-ursi Spr.	1·1	Northern Russia	*Foliae uvae ursi*	—	49·6	48·3
A. glauca Lindl.	3·2	California	—	Manizato	49·3	48·4
A. alba Nies.	3·3	U.S.A.	—	—	49·4	48·5
A. arjuma mississippiana Nies.	4·3	U.S.A	—	—	49·5	48·3
Datura arborea L.	1·2	Peru	—	—	49·6	48·5
Ourouparia rhynchophylea Mats.	0·5	Japan	—	—	49·5	48·3
(iv) Fruits:						
Caesalpinia coriaria Willd.	14·3	Mexico	—	Divi-Divi	49·6	48·5
C. brevifolia Boil.	16·2	Chile	*Balsamo carpon*	Algarobilla	49·2	48·7
C. melanocarpon var. *ugandae* Nies.	13·3	Uganda	—	Mbalimbali	49·4	48·6
Haematoxylon campechianum L.	1·2	Jamaica	—	—	49·6	48·3
Terminalia bellarica Roxb.	12·4	India	*Myrobalani bellaricae*	—	49·5	48·4
T. citrina Roxb.	28·2	India	*Myrobalani citrinae*	—	49·3	48·2
T. chebula Retz.	21·2	Nyasaland	—	Cabazoon	49·6	48·7
(v) Galls						
Terminalia chebula Retz.	11·3	India	—	Dokojo nuts	49·4	48·6
Potentilla reptans L.	0·9	England	—	Hedge bank gall	49·7	48·5
Ajuga reptans L.	0·3	England	—	Pitmaking gall	49·4	48·7
Quercus sessiliflora Sal.	1·4	England	—	Two-cell gall	49·5	48·5
(vi) Buds						
Eugenia caryophyllata Thunb.	2·2	Madagascar	*Flores caryophyllii*	—	49·5	48·6
Liriosima orata Miers.	1·1	Brazil	—	Muriapuama	49·3	48·3
Apidosperma sessiliflora Allem.	3·2	Chile	—	—	49·2	48·5
(vii) Cupalae:						
Quercus aegilops L.	28·2	Greece	—	Valonea	49·3	48·3
Q. prinus L.	17·5	U.S.A.	—	—	49·7	48·5
Quercus sp.	28·2	Uganda	—	—	49·4	48·7

* Calculated values for myrobalanitannin $C_{26}H_{28}O_{19}$: glucose, 49·4; ellagic acid, 48·5%. For further identification the samples were methylated with diazomethane and hydrolyzed; in each case tetramethyl ellagic acid was obtained, m.p. and mixed m.p. 289–290°.

† Frequently incorrectly referred to as *Companosia guavira*.

Since the original isolation of myrobalanitannin in 1910 this tannin has also been identified in witch-hazel bark (Edwards & Nierenstein, 1943), and, as shown in this communication, in a large number of other plants. As will be seen (Table 1), the bark of *T. chebula* also contains myrobalanitannin, which thus disproves the contention of Meyer (1909) that the tannin yielding ellagic acid is not present in the bark.



EXPERIMENTAL

I. *Confirmation of formula (I).* Myrobalanitannin was methylated with excess diazomethane in an apparatus similar to that described by Malkin & Nierenstein (1930) and the methanol solution refluxed for 6–8 hr. on a boiling water-bath with a solution of sulphuric acid in dilute methanol. Recrystallization of the product from methanol gave tetramethylellagic acid in microscopic needles, m.p. 289–290°. (Found: C, 60·6; H, 4·1; OCH_3, 34·2%. Calc. for $C_{18}H_{14}O_8$: C, 60·4; H, 4·0; OCH_3, 34·1%.)

II. *Distribution of myrobalanitannin.* The finely powdered materials were extracted with a mixture of chloroform and carbontetrachloride, so as to remove fats, waxes, etc., and then percolated with ethanol until no more tannin could be qualitatively detected. The residue left on evaporation of the alcohol crystallized from distilled water in faintly brownish microscopic needles, which did not melt below 360°. For myrobalanitannin Edwards & Nierenstein (1943) record $[\alpha]_D^{21°} = +21·98°$ (water); we found $[\alpha]_D^{21°} = +21·77°$ (water), $[\alpha]_D^{23°} = +37·16°$ (ethanol) and $[\alpha]_D^{17°} = +29·03°$ (methanol). The distribution of myrobalanitannin is given in Table 1. Glucose and ellagic acid were estimated by the method of Nierenstein, Spiers & Geake (1921).

SUMMARY

The original formula of myrobalanitannin has been confirmed, and the distribution of this tannin studied.

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Interfering Substances in the Roe and Kuether Method for the Determination of Ascorbic Acid

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(*Received* 4 *July* 1945)

Roe & Kuether's method (1943) is based upon the red coloration given by the 2:4-dinitrophenylhydrazine derivative of dehydroascorbic acid with 85% H_2SO_4. This reaction is very sensitive and its specificity, as will be shown, is high. We investigated the method in connexion with another inquiry and record some of our observations.